Appendix B.

Benthic Community Analysis SOP

This Standard Operating Procedure was taken from the ARCS Assessment Guidance Document (USEPA, 1994).

The first step in conducting an evaluation of benthic invertebrate communities is the development of an appropriate experimental design. There are many factors that need to be considered when sampling contaminated sediments for benthic invertebrates that differ from the considerations required for sampling sediments for toxicity testing. Benthic invertebrate distributions are strongly influenced by abiotic factors in the absence of contaminants and, in some cases, the effects of contaminants can be masked by effects due to abiotic factors. Important abiotic characteristics (e.g., sediment grain size, sediment organic content, sediment nutrient content, water quality, current velocity, water depth) at a study site should therefore be evaluated so that the potential confounding effects of these characteristics can be accounted for when the data are analyzed and interpreted.

When assessing benthic invertebrate communities for changes in community structure, it is critical to select appropriate reference sites with which the benthic invertebrate communities at study sites can be compared. Ideally, a reference site should be unaffected or minimally affected by anthropogenic influences. Since a completely unaffected system is difficult or impossible to find, it is usually considered acceptable to use sites that are considerably less contaminated than the study site. However, because low contaminant concentrations in water and sediment can sometimes affect benthic invertebrate communities, caution should be used when comparing results with a reference site that has contaminant concentrations higher than in pristine areas. A reference site should also have physical and chemical characteristics of both water and sediment that are similar to the study site to account for the potential effects of those characteristics on benthic communities at the study site.

Many studies have evaluated the number of replicate samples required to provide adequate assessments of benthic invertebrate communities and to allow cause-and-effect predictions to be made from the data. Many of these studies suggest that a sufficient number of replicate samples should be taken so that the among-sample coefficient of variation for all invertebrates is less than 50 percent. To determine the number of benthic invertebrate samples that should be collected, it is recommended that a preliminary survey be conducted of the study areas. This is done to qualitatively identify the taxa that will be encountered and the relative abundances of those taxa at each station. Depending on the types of taxa collected, the methods used to collect benthic invertebrates may need to be modified to more effectively sample the benthos.

METHODS

To minimize potential disturbance of the sediments and associated invertebrates, all

benthic grab sample should be collected before sediment samples are collected for chemistry and toxicity evaluations. The three replicate benthic grab samples at each station should be collected within a 100-m^2 area. Each benthic grab sample should be sieved through a 500-µm brass screen; site water should be used for rinsing. Material retained by the sieves will be rinsed into 500-mL glass jars and preserved with 10-percent buffered formalin.

Before sorting, samples will be rinsed thoroughly with tap water to remove formalin and excess silt or mud. The rinsed samples will be drained of excess water, returned to their original jars, and allowed to soak in 95-percent ethanol for at least 24 hours to facilitate extraction of any volatile chemicals. After the 24-hour soaking period, each sample will be rinsed again with tap water to remove the ethanol and volatile chemicals. Each sample will be placed in a 4-L wide-mouth jar and agitated with tap water so that the invertebrates and lighter detrital material floated while the snails, clams, and heavier material remained on the bottom of the jar. Aliquots of the sample will be removed from the jar to sort the benthic invertebrates from the debris. Aliquots will be removed until the entire sample had been sorted. Sorting times range from approximately 3 to 20 hours per sample.

A binocular dissecting microscope with a magnifying power of 4x-12x will be used to sort the samples. Organisms will be sorted and enumerated into the following orders or families: Oligochaeta, Chironomidae, Bivalvia, Gastropoda, Ephemeroptera, Odonata, Plecoptera, Hemiptera, Megaloptera, Trichoptera, Coleoptera, Diptera (other than Chironomidae), Hirudinea, and Amphipoda. These samples will be used to estimate macroinvertebrate numerical abundance (individuals/m^2), and taxa richness. Taxonomic identifications will be made using published taxonomic keys.

In unimpacted areas, the prevalence of deformities in chironomids is generally less than 1 percent (Wiederholm 1984; Warwick et al. 1987). Chironomid larvae will also be examined for deformities in mouthpart structures. These deformities consisted of various types of asymmetry, missing teeth, extra teeth, fusion among various teeth, and labial separation, as described by several investigators (Saether 1970; Hamilton and Saether 1971; Hare and Carter 1976; Warwick et al. 1987; Warwick 1989). Individual chironomid larvae will be mounted on slides and examined for deformities in the mentum (Orthocladinae and Chironominae) and ligula (Tanypodinae). The prevalence of mouthpart deformities will be calculated as a proportion of the total number of chironomid larvae found at each station.

Quality Assurance and Quality Control for Benthic Invertebrate Community Analysis

Benthic invertebrate samples should be collected in a manner that provides the best possible estimate of benthic invertebrate community structure. To minimize potential disturbance of the sediments and associated invertebrates, all benthic grab samples for the will be collected before sediment samples are collected for chemistry and toxicity

evaluations.

Samples will be sorted in the laboratory by a number of technicians. To ensure that all samples will be sorted with a similar efficiency, 1 of every 10 samples will be randomly selected and resorted by a supervisor to confirm that the sample was sorted completely. If the number of invertebrates found during resorting was >=5 percent of the total number of invertebrates in the sample, all 10 samples in the lot will be resorted in their entirety.

Two major elements of benthic invertebrate surveys can contribute to the variability associated with estimates of species distribution and abundance. The first is the variability associated with different field collection methods, and the second stems from inaccuracies in taxonomic identification. Although benthic community structure cannot be assessed for accuracy, precision will be monitored. The precision associated with the collection of benthic invertebrate samples will be evaluated by examining the three replicate grab samples collected at each station. The replicate samples will be collected within a 100-m^2 area at each station. The variance associated with field collection will be evaluated using an ANOVA to identify the sources of variability.

Statistical Analysis

As described by Canfield et al. (1993), data will be analyzed using appropriate parametric and nonparametric statistical tests (Snedecor and Cochran 1982). Comparisons between benthic invertebrate abundances and physical and chemical data will be conducted using correlation and multivariate regression analyses. Unless otherwise specified, statements of statistical significance refer to significance at P<=0.05.

The metrics that have showed the best ability to discriminate among sites include percent contribution of major taxa, comparisons between numerical abundances and species composition, comparisons between numerical abundances and sediment chemistry, prevalences of mouthpart deformities in larval chironomids, percent of total variance in abundance estimates, and evaluations of chironomid genera richness.

Selected data sets should be used to evaluate the following questions:

1. What is the benthic invertebrate community composition at each station?
2. Is there a significant correlation between sediment contamination and benthic invertebrate community structure?
3. Is there a relationship between larval chironomid mouthpart deformities, benthic invertebrate community composition, and physical and chemical sediment characteristics?

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